Use of succinyl chitosan as fat replacer on cake formulations

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ABSTRACT

Chitosan derivatives have been used in bakeries as dietary fiber source for obtaining healthy breads, but no other application has been suggested. This research was focused on exploring the ability of a chitosan derivative (succinyl chitosan, SC) as fat replacer in cakes. SC suspensions were used to replace different levels of fat (up to absence of fat) and the effect on batters' consistency and cakes features (color, morphogeometrics properties, texture, moisture and water activity) were evaluated. SC suspensions (2 g/100 g) were used to replace different levels of fat in cakes. By adapting batter consistencies, it was possible to obtain fat reduced batters with the same density, and cakes with high 2D area and lower ratio (width/height) containing half of the fat present in the reference cake. Crumb structure and texture features of fat reduced cakes containing SC was similar to that of full fat cakes. During staling, SC reduced hardening rate of cakes containing half fat, although drying rate was accelerated. The results indicated that succinyl chitosan has a great potential to be used as partial fat replacer on cakes, even reducing hardening rate during storage.

Keywords: chitosan; fat replacer; cake; batter; quality

1. INTRODUCTION

Sustainability is one of the main concerns of the food industry, particularly fisheries, owing to the huge amount of by-products daily generated (Martins, Pinho & Ferreira, 2017). Marine by-products from the shell of shrimp, krill, crabs and lobsters are rich in the amino-polysaccharide chitin (β (1–4) linked 2-acetamido-2-deoxy-β-d-glucopyranose), the second most abundant polymer in nature after cellulose (Alishahi & Aïder, 2012). Like cellulose, chitin is highly insoluble due to their extensive hydrogen bonding and high degree of acetylation, because of that, it is usually deacetylated for increasing its solubility leading to chitosan (β (1–4) linked 2-acetamido-2-deoxy-β-d-glucopyranose and 2- amino-2-deoxy-β-d-glucopyranose) (Pillai, Paul & Sharma, 2009). In addition, different chemical modifications of their amino and hydroxyl groups have been carried out to improve chitosan solubility, like quaternization, alkylation, carboxyalkylation and N-acylation (Bashir, Teo, Ramesh, Ramesh & Khan, 2015). Particularly, succinylation of chitosan enhances water solubility and led to a biodegradable and non-toxic material (Bashir et al., 2015). Succinyl chitosan has been reported as water soluble in acidic and alkaline media, high water retention and chelating abilities, strong antioxidant activity, apoptosis inhibitory activity and so on (Bashir et al., 2015). Because of that, succinyl chitosan has been widely used in biomedical applications.

Application of chitosan derivatives in bakery products has been focused on exploiting their health promoting properties as dietary fiber with the aim of obtaining functional foods (Kerch, Zicans & Meri, 2010). Nevertheless, Kerch et al. (2010) incorporated chitosan oligosaccharide succinate in bread and it was observed that chitosan oligosaccharides (added up to 3.6 g/110 g) prevented amylose-lipid complexation, accelerated the dehydration of both gluten and starch and increased the rate of water migration from crumb to bread crust; similarly to the effect observed with chitosan (Kerch et al. 2008), thus they cannot be used as

68 antistaling agents. However, no previous exploration of their thickening and emulsifying
69 properties has been reported on bakery products.
70 Cakes are complex bakery products that require sugar, fat and egg yolk to induce freshness,
71 soft mouthfeel and palatable textures (Karp, Wyrwisz, Kurek & Wierzbicka, 2017; Matos,
72 Sanz & Rosell, 2014). In cakes formulations, fat is used to obtain cakes with a tender, low
73 moisture, and yellow crumb. Fat functionality in cake batter is responsible of keeping gaseous
74 cells into the emulsion (oil-water) and the subsequent volume, softness and flavor of cakes;
75 besides fats extend the shelf life of the final product (Matsakidou, Blekas & Paraskevopoulou,
76 2010; Rios, Pessanha, Almeida, Viana & Lannes, 2014; Hesso et al., 2015). Owing to the
77 awareness to reduce fat content in food products and considering that bakery products are
78 consumed daily by a wide range of people, some attempts to replace the fat by different types
79 of ingredients, namely fat substitutes, have been reported (Rodriguez- Sandoval, Prasca-Sierra
80 & Hernandez, 2017). The role of fat replacers on food matrix, especially in cakes, has been
81 ascribed to their structuring properties (Psimouli & Oreopoulou, 2013; Rios, Pessanha,
82 Almeida, Viana & Lannes, 2014). Polymers like hydrocolloids have been used for the purpose
83 of improving dough-handling properties, the quality of fresh cakes and extend the shelf-life of
84 stored products. (Rodriguez- Sandoval et al., 2017; Rios et al., 2014)

85 Therefore, the aim of this research was to explore the ability of a chitosan derivative (succinyl
86 chitosan) as fat replacer in cakes. Previously, the concentration of chitosan derivative to be
87 incorporated as fat replacer was determined based on its emulsion ability (activity and
88 stability). The selected concentration of succinyl chitosan was employed to replace different
89 levels of fat and the effect of succinyl chitosan was evaluated at batter and cakes level.

90 **2. MATERIALS AND METHODS**

91 ***2.1. Materials***

92 Commercial wheat flour was supplied by Harinera La Meta S.A. (Lleida, Spain). Whole dried
93 egg was acquired from EPSA Aditivos Alimentaria Emilio Peña S.A. (Valencia, Spain).
94 Sunflower oil, sugar, skimmed dried milk and baking powder (sodium bicarbonate, tartaric
95 and malic acids) were purchased from the local market.

96 ***2.2. Methods***

97 ***2.2.1. Succinyl chitosan preparation and assessment***

98 Succinyl chitosan (SC) was obtained from *N*-succinylation reaction of chitosan using succinic
99 anhydride following the method of Mello, Bernusso, Pitombo & Polakiewicz (2006).
100 Chitosan and succinyl chitosan were characterized by Alpha infrared spectrophotometer
101 (Bruker, Germany), with ATR-FTIR (Attenuance Total Reflectance – Fourier Transformed
102 Infrared) module in the range of 4000/ cm – 600/ cm. The degree of deacetylation of chitosan
103 was calculated according to Kasaai (2010).

104 ***2.2.2. Emulsifying properties***

105 The emulsifying properties of the wheat flour and the blends of succinyl chitosan and
106 wheat flour were measured by the method of Gujral & Rosell (2004) with some
107 modifications. Blends containing succinyl chitosan at 0.5 g/100 g, 1.0 g/100 g, 2.0 g/100 g
108 and 3.0 g/100 g (flour basis) were tested. To prepare the emulsions, 2 mL of refined sunflower
109 oil and 6 mL of samples suspensions (0.005: 1 w/v) in 0.1 mol/L phosphate buffer (pH 7.0)
110 were mixed together and homogenized in a T18 Ultra Turrax (Wilmington, NC, USA) at
111 2,300 rad/s for 1 min at 20 °C. Aliquots (30 μL) of the emulsion were taken at regular
112 intervals (10 min up to 60 min) and diluted with 3 mL of sodium dodecylsulphate water
113 solution (0.001:1, v/v). The absorbance of the diluted emulsion was then determined at 500

114 nm in a spectrophotometer. The emulsifying activity was expressed as measured at 0 min.
115 Value was the average of three replicates. The emulsion stability was expressed as:

116 $ES\ (\%) = (Abs_{60\ min}/Abs_{0\ min}) \times 100$ [Equation 1]

117 ***2.2.3. Cake batter preparation and characterization***

118 Cakes were prepared as described Matos et al. (2014) with slight variations. The solids for
119 batter formulation included 300 g wheat flour; 214.4 g sugar; 32.1 g whole dried egg; 10.8 g
120 skimmed dried milk and baking powder (sodium bicarbonate 4.2 g; 2.2 g tartaric and malic
121 acids). In reference samples, 171.3 g sunflower oil was added and 350 g of water; they were
122 referred as full fat sample, where no SC was added. A solution of succinyl chitosan was used
123 as fat replacer (2 g/100 g SC, flour basis), progressively reducing the amount of fat in the
124 reference formulation. The amount of oil in fat-reduced recipes was 128.5 g, 85.65 g, 42.8 g,
125 and 0.0 g to give 25%, 50%, 75% and 100% fat reduction samples, respectively. To guarantee
126 the same batter consistency in all formulations, the amount of added water was adjusted based
127 on the batter viscosity using RVA-measurements. Whenever present, 6 g succinyl chitosan
128 were previously diluted in part of the water (171.3 g) by heating at 60 °C for 30 min. Total
129 water added to each recipe were 348 g, 365 g, 390 g, and 421 g of water to obtain 25%, 50%,
130 75% and 100% fat-reduced batters, respectively.

131 In a RM8 mixer (Robot Coupe U.S.A., USA), all of the dry ingredients, except the baking
132 powder, were pre-mixed for 1 min for complete homogenization. Thereafter, the sunflower oil
133 and / or SC (depending on the formulation) were added, starting the mixing. During the
134 mixing process, the water was gradually incorporated until a homogeneous batter was
135 obtained; finally, the baking powder was added at the end of the process. The total mixing

time was 5 min until getting a spongy batter. Part of the batter (50 mL) was used for further determining density and pasting properties. Two batches were carried for each recipe.

The density of the batter was measured as the ratio of the weight of a graduated cylinder filled with batter to that one filled with water. Three replicates were determined.

The pasting and viscosity properties of the batter were measured using a Rapid Visco™ (RVA 4500, Perten Instruments, New South Wales, Australia) as previously described (Xing, Niu, Su & Yang, 2016) to record the changes of a complex system like batters when transforming from a mixing suspension to a gel-like structure. Eight grams of batter were diluted in 12 g of distilled water inside of an aluminum canister and maintained at 50 ºC for 1 min, then heated from 50 to 95 °C at 12 K/min and held at 95 °C for 1 min. After that, the batter was cooled at 12 K/min and held at 50 °C for 2 min. The mixing speed was 63 rad/s and the viscosity was monitored (mPa.s). The experiment was conducted twice per sample. Pasting parameters such as onset temperature, peak viscosity, trough, breakdown (peak viscosity-trough), final viscosity, setback (final viscosity-trough) were recorded using Thermocline software for Windows (Perten Instruments, Hägersten, Sweden).

2.2.4. Cakes preparation and evaluation

Batter prepared as described in section 2.2.3 was poured into the disposable bags and 30 g were dispensed in each cake paper cup (45 mm x 27 mm) and baked in a conventional oven model Labe (Salva Industrial S.A., Gipuzkoa, Spain) for 12 min at 180 °C. Cakes were cooled down at room temperature during 90 min. Fresh cakes were characterized for texture, color, image, water activity and moisture content. Part of the cakes were packed in 15 cm x 30 cm polyethylene plastic bags and stored in a temperature controlled chamber at 25 °C (Gabarro,

158 Barcelona, Spain) for staling study during seven days. Two batches were made for each
159 formulation on different days.

160 Texture was determined in fresh and stored cakes (at days 0, 2, 4 and 7 after baking). The
161 texture profile analysis of the cake crumb was made with a TA.XT.plus Texture Analyzer
162 (Stable Microsystems, Godalming, UK) provided with Texture expert software. Cakes were
163 horizontally cut giving a section of 1.5 cm thickness. A double compression test (texture
164 profile analysis) was performed with a 75 mm diameter flat-ended cylindrical probe (P/75)
165 and compression to 50% of the initial height at a speed of 1 mm/s with 5 s waiting time
166 between the two cycles. Parameters recorded included: hardness, springiness, cohesiveness,
167 chewiness and resilience. Results were the mean values of at least four replicates for each
168 sample. Moisture content was determined in fresh and stored cakes (at days 0, 2, 4 and 7 after
169 baking) following the two-step oven method of the AACCI method 44-15A. The water
170 activity was measured in an Aqua Lab model 3T (Decagon Devices, Inc., USA). Hardening
171 rate (g/day) and drying rate (g/100 g per day), during the 7-days storage, were calculated
172 using the hardness increase or moisture content decrease, respectively.

173 Color was determined on cake crumb using a Konica Minolta CM-3500 spectrocolorimeter.
174 Illumination (D65) with a 10° viewing angle and a 30 mm port size was used. The CIE-
175 $L^*a^*b^*$ values of cake crumb were recorded: L^* (lightness/darkness), a^* (redness/greenness),
176 and b^* (yellowness/blueness). The measurements were made in three different regions on the
177 surface of the crumb previously cut from the central part.

178 Fresh cakes were cut in longitudinal and transversal mode. High resolution images (600 dpi)
179 of the cakes were captured by HP Scanjet G3110 (Hewlett- Packard, USA). A single 30 x 30
180 mm square field was cropped and analysed by Fiji Image J software (Schindelin et al. 2012)
181 for each image. Color channels were separated to improve differences between cells and

crumb, the pre-defined "otsu" algorithm were aplied to establish de threshold and then particles were analyzed. The studied parameters were mean cell area (mm^2), cell density or number of cells for cm^2 (cell/cm^2), surface porosity (calculated as total cell area/cross section area, expressed in percentage) and cell circularity (from 0 (square) to 1 (perfect circle)). To study the whole morphogeometric characteristics of cakes, width and height, 2D area (mm^2), 2D perimeter (mm) and the ratio (width/height) of each slice were calculated using the same software.

2.2.5. Statistical analysis

In order to assess significant difference among samples, a one-way ANOVA analysis of samples was performed using the program Statgraphics Centurion XVI Version 16.2.04. Fisher's least significant differences (LSD) test was used to describe means with 95% confidence.

3. RESULTS AND DISCUSSIONS

3.1. Succinyl chitosan characterization on ATR-FTIR and emulsifying properties

The deacetylation degree of the chitosan used was 77.0%, based on the peak area values of the absorption bands at 1655/cm and 3450 /cm that are associated with carbonyl and hydroxyl groups, respectively (Kasaai 2010). The calculated ATR-FTIR results for chitosan and succinyl chitosan are presented in Table 1. The main difference of the spectra was displacement of the absorption band observed at 1568/cm attributed to amide II and the carbonyl stretching (C=O) at 1635/cm. Also, the presence of the symmetric stretching (ν_s) of carboxyl group $\nu_s(COO^-)$ is a direct evidence of substitution, as well as absent in the chitosan structure, and confirms the presence of the succinated group.

204 The effect of different concentrations of succinyl chitosan on the emulsifying activity (EA)
205 and emulsifying stability (ES) are summarized on Table 2. As expected, EA of the blends
206 increased with the SC concentration, reaching a maximum when containing 2 g SC /100 g
207 flour. The emulsifying stability also increased in the presence of SC, but only in the range 1-2
208 g/100 g concentration. It has been reported that functionality of other biopolymers changes
209 when increasing concentration due to phase separation processes as has been observed with
210 rice starch and ionic hydrocolloids (Rosell, Yokoyama & Shoemaker, 2011). Presumably,
211 only up to 2 g/100 g SC is needed to decrease the superficial tension and to maintain the
212 emulsion stable for a long period. Therefore, the emulsifying properties suggested the use of 2
213 g SC /100 g flour basis in solution (in aqueous medium) the most adequate to stabilize
214 biphasic systems.

215 ***3.2. Effect of succinyl chitosan on pasting properties and density of the cake batter***

216 The addition of 2 g SC /100 g flour was used to obtain reduced fat batters. With that purpose,
217 batters with different levels of oil were prepared in order to determine the amount of fat that
218 the SC was able to replace. At the same time, hydration was individually adapted for each
219 batter to keep a steady consistency when decreasing fat levels, because it is already known
220 that excessive batter consistency might limit its expansion (Gularte, de la Hera, Gómez &
221 Rosell, 2012).

222 Viscosities of the batters were compared during a heating-cooling cycle and pasting
223 parameters were determined (Table 3). It is well stated that fats affect the properties of starch
224 during gelatinization and retrogradation mainly due to the complex formation of lipids with
225 starch, namely amylose, which produces an increase in the breakdown (Gujral & Rosell
226 2004). Accordingly, the batters containing different percentages of fat reduction (25, 50, 75,
227 and 100%) showed significant differences in the resulting breakdown of the batters, compared

228 to the batter with full fat content (0% reduction), but no significant differences ($P > 0.05$)
229 were observed on onset temperature, peak viscosity, trough, final viscosity and setback
230 parameters. Regarding breakdown, a significant decrease was observed up to 50% fat
231 reduction, but beyond that level a progressive increase was detected (Table 3). Fats increase
232 the breakdown related to the stability of the starch during cooking, thus it was expected that a
233 decrease in the fat content induces a decline in this parameter. Nevertheless, it seems that at
234 higher fat reduction (> 50%), strong interaction between the SC structure and the starch
235 molecules was produced stabilizing the integrity of the starch granule during heating, as has
236 been described for ionic hydrocolloids like xanthan gum (Diao et al. 2017; Rosell et al., 2011)
237 Anyway, interactions of starch with hydrocolloids during gelatinization lead to different
238 network structures, whose pasting and rheological properties depend on the hydrocolloid and
239 its concentration (Diao et al. 2017; Shi & BeMiller 2002). Chitosan-containing emulsion was
240 found to be dependent on molecular weight, concentration and deacetylated degree
241 contributing to the viscosity enhancement (Klinkesorn, 2013; Kerch et al., 2008). Therefore,
242 the SC with 77% degree of deacetylation showed a behavior like ionic hydrocolloids.

243 Batter density was assessed (Table 3) and no changes were observed due to fat reduction in
244 the presence of SC. Similar density values indicated comparable amount of air incorporated
245 into the batter, which was ascribed to the consistency adjustment previously carried out.
246 Conversely, Psimouli & Oreopoulou (2013) reported that the addition of carbohydrate-based
247 compounds (maltodextrin, oligofructose, pectin, inulin and microparticulated whey protein
248 concentrate) to replace 35, 65 or 100% of fat on cake batter significantly increased batter
249 density and decreased viscosity, likely due to the different fat replacer structure and recipes.
250 Nevertheless, in contrast with the present study, those authors did not adjust the water content
251 of the recipes to guarantee the same batter viscosity.

3.3. Effect of fat reduction on morphogeometrics characteristics and colour parameters of fresh cakes

The fat reduction by adding succinyl chitosan confers marked differences in morphogeometrics characteristics of the cakes (Table 4) particularly significant in 2D area and ratio width/height. Variations in 2D area, perimeter and ratio were observed on the cross-section of cakes in Figure 1. In general, the reduction of fat content gave high volume cakes (high 2D area); although in the case of 75% reduction was not significant ($P > 0.05$). This result was opposite to those obtained when adding chitosan to bread, where either a volume decrease (Lafarga, Gallagher, Walsh, Valverde & Hayes, 2013) or no significant effect was observed (Kerch et al., 2010), depending on the chitosan derivative. Hence, results suggested a proper functionality of chitosan derivative as fat replacer in cakes but no as improver in bread. The improving effect of SC was also observed in the reduction of the ratio width/height (Table 4, Fig. 1), but only up to 50% fat reduction. Despite the similar viscosity encountered on the batters during heating (Table 3), some significant changes were observed on the shape and volume of the cakes, suggesting that SC affected the gas incorporation into the batter, and also the ability of the structure to expand during baking.

Regarding crumb color, the addition of SC significantly modified the luminosity (L^*) and slightly the redness tonality (a^*). In general, SC-containing cakes exhibited significantly lighter crumb and no trend was observed with the level of fat reduction.

Internal structure of the cakes was evaluated by digital analysis (Table 4) and results confirmed that no significant effect was observed on either, cell density, mean cell area or surface porosity when fat content was reduced in SC containing cakes. As expected, constant batter consistency ensures the same air incorporation obtaining cakes with similar cell density and cells size. Only significant effect was observed on the circularity of the internal cells, the

reduction of fat content up to 50% yielded rounded gas cells. That result agrees with observation on batter pasting properties, supporting that SC contributes to the integrity of the starch granules and their stability during cooking when sufficient reduction of fat was produced, which could favor expansion in more uniform cells. The properties of SC contribute to the performance of the porosity characteristics (Klinkesorn, 2013). But it seems that in excess of water (with higher fat reduction) limited interactions between starch and SC were produced due to starch dilution or water molecules competing with SC for interacting with starch.

In parallel to the fat reduction, an increase in the amount of water was carried out to keep batters' consistency. Consequently, the cake moisture content showed a progressive increase with the fat reduction (Table 4). The presence of SC in the cakes did not modify the amount of free water available in the crumbs, as has been described when 2 g/100 g of chitosan was added in breads (Kerch et al., 2008). In fact, the 25% fat reduction, which hardly require a change in the amount of water (348 ml vs 350 ml in control), led to similar moisture content to that obtained in the full fat cake (0%). Nevertheless, significant changes were observed in the water activity (a_w) (Table 4). A decline in the a_w was observed in the cakes that were made with up to 50% fat reduction, likely due to the water binding capacity of SC (Lafarga, 2013). However, the maximum fat reduction (100%) resulted in higher a_w than the full fat cake.

3.4. Effect of fat reduction on textural parameters of SC-containing cakes

When the level of fat was reduced in the cakes containing succinyl chitosan, significant changes were observed in the texture related parameters, with exception of springiness (Table 5). The complete removal of fat (100% fat reduction) resulted in the softest cake and no changes were detected with the other reduction levels, compared to the full fat cake. Previous studies carried out with breads reported that the addition of chitosan increased the firmness of

300 bread crumb and in the case of chitosan oligosaccharides the effect depended on the
301 oligosaccharide molecular weight (Kerch et al., 2010). Considering cohesiveness and
302 resilience, the progressive fat reduction induced a steady increase of these parameters, which
303 are indicative of food structure with higher ability to recover after compression (Matos et al.,
304 2014). Usually, those have been associated to crumbs with higher number of gas cells, but
305 taking into account that crumb porosity was similar in all the cakes, the result obtained might
306 be attributed to the plasticizer role of the water molecules in the fresh cakes. In fact, during
307 storage of the cakes, when a significant dehydration was observed (Table 5), the texture
308 behaved significantly different. Hardening rate of SC-containing cakes decreased, but only up
309 to 50% fat reduction cake. Above this percentage (75% and 100% fat reduction), an opposite
310 tendency was observed. Therefore, SC was able to act as anti-staling agent in cakes and
311 replacing up to 50% the fat content of the cakes. The use of chitosan and its derivatives have
312 been solely studied in bread formulations, which are quite distinct. In breads, chitosan is
313 adsorbed onto the starch surface preventing the amylose-lipid complexation and accelerating
314 the aging of bread (Kerch et al. 2008; Rakcejeva, Rusa, Dukalska & Kerch, 2011) and
315 increasing crumb hardening (Lafarga et al. 2013). Based on that, present results might be
316 attributed to its synergy with the lipids portion of the formulation, since SC could act as an
317 amphiphilic surfactant (hydrophilic and hydrophobic groups) (Bashir et al., 2015) capable of
318 binding fats, besides proteins and polysaccharides presents in the cakes recipe.

319 In addition, results on the drying rate (Table 5) during the storage time, showed higher values
320 in cakes with reduced fat, with the exception of non-fat cake (100% reduction). The drying
321 rate decreased when increasing the level of fat reduction, thus the presence of SC speeded up
322 the release of water molecules. It seems that interaction of SC with fat structure accelerated
323 the drying during storage, which was partially compensated with the water increase for batter
324 consistency adjustment. But in the absence of fat, SC reduce the drying, likely due to chitosan

retards the redistribution of water between the bread components (gluten and starch) although at the same time accelerates the dehydration of gluten and starch and the migration of water from the crumb to the crust (Kerch et al. 2008; Rakcejeva et al., 2011).

4. CONCLUSIONS

Succinyl chitosan, a water soluble biopolymer, with 77% degree of the deacetylation increased the emulsifying capacity of wheat flour and that effect was maximum when 2 g SC /100 g flour was blended with flour. In consequence, the ability of SC as fat replacer was checked in a bakery product with high fat content like cake. Batters of comparable consistency were obtained with different levels of fat content (0-100%) in the presence of 2 g SC /100 g flour. When producing fat reduced cakes, SC (2 g/100 g) was able to replace up to 50% of the cakes fat content, without altering batter pasting properties, only breakdown. Crumb structure of fat reduced cakes containing SC was similar to that of full fat cakes, even SC improved cell circularity. Regarding texture properties, effect of SC was readily evident during cake storage, reducing hardening rate, although drying rate was accelerated. Succinyl chitosan could be considered a potential fat substitute for cake formulation, although more investigations regarding consumer acceptance should be carried out.

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Table 1. ATR-FTIR results of chitosan and succinyl chitosan.

Group characteristic	Chitosan (wave number 1/cm)	Succinyl chitosan (wave number 1/cm)
ν(OH),(NH)	3369 s	3381 s
ν(CH)	2910 m	2918 m
Amide II	1568 w	1556 s
ν(C=O)	1635 vw	1646 m
$\nu_s(COO^-)$	-	1401 s
Saccharide backbone	1152 vs	1068 vs

s = strong; vs = very strong; m = medium; w = weak; vw = very weak.

Table 2. Emulsifying and foaming properties of wheat flour-succinyl chitosan blends.

Samples	EA (AU)	ES (%)
wheat flour	0.12 ± 0.05	37.8 ± 1.4
SC 0.5%	0.51 ± 0.13	20.3 ± 5.0
SC 1.0%	0.56 ± 0.05	46.4 ± 5.6
SC 2.0%	0.80 ± 0.01	62,6 ± 6.7
SC 3.0%	0.60 ± 0.02	39.5 ± 2.8

EA = emulsion activity; ES = emulsion stability;
SC = succinyl chitosan. AU = absorbance units (nm).
Values are given as mean (n = 3) ± standard deviation.

Table 3

Effects of succinyl chitosan on pasting properties and density of cake batters obtained with different levels of fat reduction.

Fat reduction (%)	Onset (°C)	Peak viscosity (mPa.s)	Trough (mPa.s)	Breakdown (mPa.s)	Final viscosity (mPa.s)	Setback (mPa.s)	Batter density (g/mL)
0	65.72 ± 0.40	1363 ± 165	580 ± 40	858 ± 110^{c}	1103 ± 64	548 ± 4	1.10 ± 0.10
25	65.76 ± 0.66	1301 ± 41	529 ± 11	758 ± 22^{ab}	1124 ± 21	595 ± 19	1.00 ± 0.03
50	65.72 ± 0.36	1282 ± 26	532 ± 18	749 ± 34^{a}	1138 ± 28	608 ± 23	0.98 ± 0.03
75	65.92 ± 0.81	1365 ± 91	549 ± 63	816 ± 38^{bc}	1141 ± 101	592 ± 46	1.06 ± 0.10
100	65.53 ± 0.38	1358 ± 64	526 ± 30	832 ± 46^{c}	1135 ± 62	609 ± 36	0.93 ± 0.07
P-value	*0.8852*	*0.3262*	*0.4736*	***0.0204***	*0.893*	*0.1432*	*0.2902*

Values are given as mean ± standard deviation. (n = 4). Same letter within a column are not significantly different ($P > 0.05$) for each specific parameter.

Table 4
Effect of fat reduction on shape and crumb color of succinyl chitosan containing cakes.

Fat reduction (%)	Crumb morphogeometric characteristics			Crumb colour		
	2D area (cm^2)	2D Perimeter (cm)	Ratio (width/height)	*L**	*a**	*b**
0	13.83 ± 1.54^{a}	30.07 ± 5.78	1.31 ± 0.07^{b}	61.40 ± 3.53^{a}	-2.65 ± 0.23^{ab}	19.95 ± 3.26
25	17.56 ± 0.54^{bc}	30.05 ± 2.18	1.09 ± 0.03^{a}	68.65 ± 2.52^{c}	-2.51 ± 0.25^{b}	$20.73 \pm 0,71$
50	19.05 ± 1.19^{c}	29.35 ± 2.93	1.02 ± 0.04^{a}	65.18 ± 2.98^{b}	-2.42 ± 0.29^{bc}	19.98 ± 1.34
75	14.51 ± 1.62^{ab}	30.40 ± 10.41	1.32 ± 0.14^{b}	64.05 ± 2.08^{b}	-2.28 ± 0.16^{c}	21.25 ± 1.37
100	18.35 ± 4.85^{c}	24.18 ± 2.46	1.29 ± 0.10^{b}	64.81 ± 2.32^{b}	-2.72 ± 0.30^{a}	21.26 ± 0.93
P-value	***0.0272***	*0.4587*	***0.0004***	***0.0000***	***0.0009***	*0.3680*
	Crumb structure				Physicochemical parameters	
Fat reduction (%)	Cell density ($cell/cm^2$)	Mean cell area (mm^2)	Surface porosity (%)	Circularity	Moisture content (g/100 g)	Water activity
0	9.58 ± 1.40	1.78 ± 0.20	16.78 ± 1.21	0.63 ± 0.04^{a}	32.28 ± 0.96^{a}	0.931 ± 0.012^{bc}
25	7.33 ± 1.58	2.25 ± 0.70	15.65 ± 1.76	0.70 ± 0.02^{b}	32.69 ± 0.52^{a}	0.925 ± 0.004^{b}
50	9.47 ± 1.36	1.87 ± 0.50	17.18 ± 2.28	0.71 ± 0.02^{b}	35.13 ± 0.80^{b}	0.911 ± 0.011^{a}
75	8.75 ± 0.62	2.13 ± 0.17	18.61 ± 1.82	0.66 ± 0.04^{ab}	37.07 ± 1.85^{b}	0.938 ± 0.009^{cd}
100	8.06 ± 0.86	2.01 ± 0.07	16.14 ± 1.15	0.62 ± 0.04^{a}	40.81 ± 3.72^{c}	0.943 ± 0.005^{d}
P-value	*0.2164*	*0.5767*	*0.2644*	***0.0115***	***0.0000***	***0.0000***

Values are given as mean ± standard deviation. ($n = 4$). Same letter within a column are not significantly different ($P > 0.05$) for each specific parameter.

Table 5
Effect of fat reduction on crumb texture and the rate of hardening and drying of succinyl chitosan containing cakes.

Fat reduction (%)	Hardness (N)	Springiness (-)	Cohesiveness (-)	Chewiness (g)	Resilience (-)	Hardening rate (N/day)	Drying rate (g/100 g per day)
0	130 ± 10^{b}	0.981 ± 0.012	0.724 ± 0.013^{a}	922 ± 71^{a}	0.307 ± 0.009^{a}	27.8	3.85
25	132 ± 7^{b}	0.993 ± 0.015	0.758 ± 0.010^{b}	1029 ± 72^{a}	0.308 ± 0.009^{a}	26.1	5.64
50	130 ± 10^{b}	1.009 ± 0.051	0.759 ± 0.026^{b}	985 ± 37^{a}	0.303 ± 0.015^{a}	14.0	5.00
75	129 ± 11^{b}	1.007 ± 0.037	0.798 ± 0.017^{c}	1186 ± 90^{b}	0.332 ± 0.020^{b}	35.4	4.00
100	111 ± 4^{a}	0.997 ± 0.012	0.862 ± 0.031^{d}	960 ± 23^{a}	0.432 ± 0.022^{c}	49.2	3.46
P-value	***0.0003***	*0.0697*	***0.0000***	***0.0095***	***0.0000***	-	-

Values are given as mean ± standard deviation. (n = 4). Same letter within a column are not significantly different ($P > 0.05$) for each specific parameter.

Figure 1.

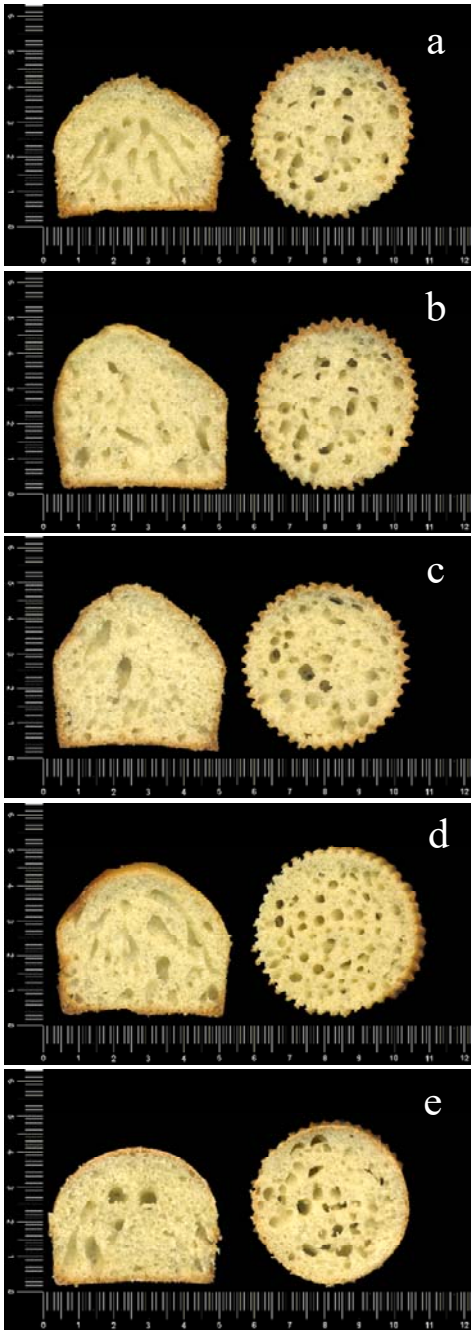

Fig. 1 Captured images (600 dpi) of the longitudinal and cross section of the succinyl chitosan containing cakes obtained with different levels of fat reduction: a: 0%, b: 25%, c: 50%, d: 75%, e: 100%.Two batches were carried out for each sample.